INTRODUCTION

Forensic Toxicology is one of the branches of forensic Science in which toxicological analysis of various biological samples is carried out to detect the presence of drug intake by users of any toxic substances present. It is found that the detection window of the drug abuse in blood and urine can only be detected within the time limit of 4-6 days. To overcome this situation alternative specimen are needed to be examine. However hair samples could solve the problem of this nature and time limit can be increased from days to months/week. But it has been realize that the quantity/ concentration of the metabolites of the drug in hair are found either low or not sufficient to detect even with the modern technology. Therefore the significance of toenails and fingernails analysis has become an alternative and reliable technique as that can examine metabolite even after years. The quantity of the material as evidence is also found sufficient for the use of this technology. [1, 3, 4]

Nail is one of the physical evidences that can be encountered in criminal cases related to homicide and sexual offences etc. Incorporation of substances into the nails mainly occurs through diffusion of compound from the rich blood supply, which accumulate substances to both germinal matrix and the nail bed on the underside of the nail plate, thus allowing incorporation in both a vertical and horizontal way during nail formation. Unlike other type of biological evidence, nail is remarkably stable to most of the environmental conditions and does not break down easily. So this study has been proposed. [5]

REVIEW OF LITERATURE

Gupchup GV et al (1999) reviewed structural characteristics and permeability properties of the human nail. In this review nail penetration has been reported to be affected by molecular size and hydrophilicity; smaller, water-soluble molecules are found to preferentially permeate the nail. Permeation of undissociated drugs is favored in certain instances. They also indicated that the nature of the vehicle can influence drug penetration. Recent review research has focused on improvement of penetration of topically applied actives into and through the nail. Studies have shown that compounds containing sulfhydryl groups in conjunction with keratolytic agents can significantly enhance drug penetration, relative to a control formulation (without enhancer). Such sulfhydryl compounds are thought to reduce the disulfide linkages in the nail keratin matrix. Thus, although some success has been achieved in enhancing penetration of drugs through the nail, they suggested that further research is required to achieve successful topical products for treatment of nail infections.[1]

Nikolaos P. Lemos et al (1999) detected and quantified cannabinoids from fingernail clippings. Specimens were obtained from consenting adults attending a drug clinic, along with information concerning the drugs which they had used over the previous six months. Analysis performed by radioimmunoassay (RIA) and gas chromatography-mass spectrometry (GC-MS) to detect and quantitate cannabinoids present in fingernail clippings.[2]

Palmeri et al (2000) reviewed physiology, pharmacokinetics and forensic toxicology of drug in nails. They reviewed mechanism of drug incorporation into nails, various diseases related to nails and post mortem detection of nail in forensic toxicology.[3]

Daniel et al (2004) reviewed drug and toxins detection from hair and nails and their application in general and forensic medicine. They discussed advantages of analyzing hair and nail samples which include their easy and non-invasive collection, the small sample size required for analysis, and their easy storage at room temperature.[4]

Katarzyna Anna Madej (2010) reviewed various methods for determination of medicines and drugs of abuse in three biological specimens – meconium, nails and tears – based on the literature since 1998. General descriptions of specimens, sample-collection methods and sample-preparation procedures were described in this review. [5]

Jin Young Kim et al (2012) developed a rapid and simple gas chromatography–mass spectrometry (GC–MS) method for the simultaneous detection and quantification of five psychotropic phenylalkylamines (amphetamine, methamphetamine, 3, 4 methylene dioxy amphetamine, 3,4-methylenedioxymethamphetamine, and norketamine) in toenails. After external decontamination, nail clippings were mechanically pulverized with a bead mill and then incubated in methanol under ultrasonication at 50_C for 1 h. The resulting solutions were evaporated to dryness, derivatized, and analyzed by GC–MS. The intra- and inter-day precisions were within 10.7% and 13.9%, respectively. The intra- and inter-day accuracies were) 4.2% to5.0% and) 2.4% to 8.4%, respectively. Limits of detection and quantification for each analyte were lower than 0.024 and 0.08 ng／mg, respectively. The recovered were in the range of 80.6–87.5%. The results indicated that the proposed method is a simple, rapid, accurate, and precise for quantification of five phenyl alkylamines in nails. Their method was successfully applied to the simultaneous detection and quantification of phenylalkylamines in nail samples of possible drug abusers.[6]

Joseph Jones et al (2013) fully validated 2-dimensional gas chromatography-tandem mass spectrometry method for the detection of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCA), the chief metabolite of the main psychoactive compound in marijuana, Δ9-tetrahydrocannabinol (THC) studied and evaluated the usefulness of fingernail clippings as an alternative specimen type to hair by the analysis of a set of 60 matched pairs of head hair and fingernail clippings. The limit of detection was reported 10 fg/mg, the limit of quantitation was 20 fg/mg, and the assay was linear from 20 fg/mg to 500 fg/mg. The intra- and inter-assay imprecision and bias studies at 4 different concentrations (50, 100, 500, and 1000 fg/mg) were

acceptable where all % Target observations were within 16% of their expected concentrations and all %CV calculations were less than 13.5%. THCA was detectable in more fingernail specimens (53.3%) than hair specimens (46.7%) and the mean concentrations in nails were on average 4.9 times higher than in hair (1813 fg/mg and 364 fg/mg, respectively). The THCA concentrations in hair and nail were strongly associated ($r = 0.974$, $P < 0.01$, $n = 60$) and the association was significant. This study demonstrated that fingernail clippings are a suitable alternative specimen type to hair to monitor for marijuana use and abuse.[7]

Giorgia De Paoli et al (2013) developed and validated liquid chromatography (HPLC) electrospray tandem mass spectrometry and ultraviolet (UV) detection method for the determination of three phencyclidines such as 2-(ethylamino)-2-(3-methoxyphenyl) cyclohexanone (methoxetamine), N-ethyl-1-(3-methoxyphenyl) cyclohexanamine and 1-[1-(3-methoxyphenyl) cyclohexyl] piperidine compounds in blood, urine and vitreous humor.[8]

Franziska Krumbiegel et al (2014) studied the usefulness of nail samples instead of hair for a general unknown screening (GUS) of drugs. An alternative matrix for long term detection is still needed in cases where no hair is available for analysis. In their study Hair and nail samples from 70 postmortem cases were analyzed by liquid-chromatography quadrupole time-of-flight mass spectrometry. Hair and nail samples were ground by a ball mill and extracted twice for 18 h. Extracts were measured in Auto MS/MS mode. Resulted only 10 % of the cases showed a disagreement in hair and nail analysis where hair samples were tested positive and corresponding nail samples were tested negative in a general unknown screening for drugs. In most of the cases investigated the analysis of the nail clippings and whole nail samples led to results comparable to those obtained from hair analysis. Incorporation of a large number of substances into the nail matrix was proven by the detection of 89 different analytes.[9]

Franziska Krumbiegel et al (2016) described the validation of multi-target methods for the determination of 76 different analytes in hair and nail samples. They have taken segmented hair and nail samples from autopsy cases. Drugs of abuse,

psychotropic drugs, and other drugs were included for the validation of this method. Hair and nail samples were ground using a ball mill and extracted for 18 h. in their study extracts were measured using a UHPLC-triple quadrupole-mass analyzer. Analytes were separated on a RP 18 column under gradient elution of the mobile phases, water with 0.1 % formic acid and acetonitrile. Whole nail and hair samples from seven autopsy cases were split into segments and analyzed. The entire method was validated according to the German Society of Forensic Toxicology guidelines. They also determined the concentration ratios of selected substances and their metabolites. [10]

Triniti L. Scroggin et al (2017) developed a method for the detection and quantification of cocaine and metabolites (cocaethylene, benzoylecgonine and meta-hydroxy benzoylecgonine), phencyclidine (PCP) and barbiturates (Phenobarbital and butabarbital) in meconium using quantitative polarity switching liquid chromatography coupled with a tandem mass spectrometer (LC–MS/MS) method.[11]

RATIONALE

Forensic onychology is the study of fingernails and toenails which plays significant role in criminal justice system. Nails are important tissues for human identification and their keratinized matrices to detect illicit drug of abuse and previous drug intake. The main biological function of nails is as protection for the underlying tissue of the nail bed. Several studies have been carried out for the detection of drug of abuse of narcotics and depressant from nails. Some authors have also reviewed about systematic incorporation of drugs into nail, their extraction and analysis using techniques like RIA, GC-MS, LC-MS, and LC-MS-MS etc. In the view of significant role of forensic onychology, further studies are expected to explore its area. However studies have been made in this field but especially little work have been reported with special reference to hallucinogens. In the proposed work efforts will be made to extend detection of hallucinogens from fingernails and toenails, to improve Methodology e.g. extraction methods for the detection of drug of abuse in fingernails and toenails and to know the previous drug intake.

OBJECTIVES

Nails are important tissues for human identification and their keratinized matrices to detect illicit drug abuse, previous drug intake or any kind of other toxic substances. Therefore, the proposed study has been undertaken as an alternative matrix for long term detection of past drug intake.

The objective of the present study is to detect various hallucinogens and evaluate its analytical limits of long- term follow up of consumption and this study is divided into as follows.

1. Extraction and characterization of hallucinogens from fingernail and toenail samples.
2. To study qualitative and quantitative analysis of drug of abuse from fingernail and toenail samples.
3. Comparison of drug concentration in fingernail and toenail samples.

MATERIAL AND METHODOLOGY

Study design:

The Study Design is summarized as below:

1. Sample collection
2. Sample decontamination
3. Extraction of analytes from the matrix (i.e. toenails and fingernails)
4. Clean up
5. Detection
6. Qualitative and quantitative interpretation of the results.

- **Sample collection: -** samples will be collected with the consent of the subjects from street areas, hospitals and rehab centers of the Madhya Pradesh.

- **Sample decontamination: -** Decontamination will be performed as per the standard procedures with reference to the analytes to be examined.

- **Preparation and Extraction:** Extraction of the analytes will be carried out with reference to the solubility of the analytes.

- **Clean-Up:** as per standard procedure
    1. Solid phase extraction.
    2. Liquid phase extraction.

- **Detection -** analysis of the hallucinatory drugs will be performed using biochemical, chromatographic and spectroscopy methods such as gas chromatography -mass spectrometry, High performance liquid chromatography, liquid chromatography – mass spectrometry and radio immunoassay method etc.

- Qualitative and quantitative interpretation of the results will be performed as per the technique to be applied.

EXPECTED OUTCOME

Analysis of nails will be very useful because keratinized matrix like fingernails and toenails are capable to accumulate drugs for long term exposure. The study will be fruitful and encouraging as well as have several advantages over the conventional matrices such as blood and urine, including a longer detection window (month to year), non invasive sample collection, easy storage, and nail can be preserved even after skeletanized remains. Thus these are the some interesting aspects make nails significant matrices for forensic toxicology, clinical toxicology and forensic pathology.

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TENTATIVE CHAPTER SCHEME

Chapter – 1

Introduction

Chapter – 2

Review of literature

Chapter – 3

Material and Methodology

Chapter - 4

Results

Chapter - 5

Discussion

Chapter – 6

Summary

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Conclusion

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Suggestion

Chapter – 9

Implication of study

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Appendix

References